UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY OF HAEMORRHAGIC SEPTICAEMIA IN CATTLE AND BUFFALOES IN PENINSULAR MALAYSIA

KHADAK SINGH BISHT

FPV 2007 4

DEDICATED WITH LOVE AND GRATITUDE

TO

MOTHER: DURA DEVI BISHT

WIFE: MAHESWARI BISHT (MISU)

SONS: BIRAJ BISHT AND BISHAL BISHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree Doctor of Philosophy

EPIDEMIOLOGY OF HAEMORRHAGIC SEPTICAEMIA IN CATTLE AND BUFFALOES IN PENINSULAR MALAYSIA

By

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June 2006

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A retrospective study on haemorrhagic septicaemia (HS) was conducted through a questionnaire survey in 64 districts of Peninsular Malaysia. One thousand four hundred and eighty nine deaths (1489) due to HS were reported from January 1993 to December 2003. Outbreaks of the disease were reported almost every year despite many precautionary measures taken. Out of the eleven (11) states surveyed in Peninsular Malaysia, HS was identified as endemic in Terengganu, Kelantan and Perak while the remaining states were considered as no disease. Time series seasonal decomposition method distinguished the patterns (seasonal, secular trend, cyclic and irregularity fluctuations) of the HS occurrence and its relationship with the climatological pattern (rainfall, temperature and humidity), while movement of animals during the main festive seasons and vaccination was also described.

Two hundred and four buffaloes (204) and four (4) cattle died of HS in Batang Padang, Perak in 2003. An epidemiological investigation was performed during the

outbreak where clinical samples were collected, farmers were interviewed and field visit was made. *Pasteurella multocida* B:2 was isolated and identified from both the heart blood and nasal swabs of the affected animals. The buffaloes that died in the pond in the grazing area played a major role in the rapid spread of the disease. The explosive outbreak was due to a combination of factors such as introduction of the healthy carriers from the endemic areas, significant climatic changes and low immune status of the susceptible herds.

Development of an ELISA test system for HS diagnosis was validated based on samples from both HS infected and uninfected populations. An area under receiver operating characteristic (ROC) curve showed that the test was highly accurate, separating the population into two different disease status groups. The cut-off value obtained by the ROC analysis for indirect ELISA gave 86.4% diagnostic sensitivity and 84.2% diagnostic specificity, based on 0.51 OD cut-off point.

The status of cattle as carriers of *P. multocida B:2* was investigated in three bovine herds in the no disease areas and three bovine herds in the endemic areas. A total of 186 animals from the selected farms were selected and followed for three to five consecutive times over a period of six months to determine their status as carriers of the HS-causing organism. Isolation of the organism was performed using mice and the serum antibody was detected using indirect ELISA. Bacteriological analysis did not reveal any of the sampled animals to harbour the HS-causing organism at any point during the 6-month study period. However, some level of immunity appeared to be existed within these populations. The mean optical density (OD) values in the no disease areas were lower than the mean OD values in the endemic areas ($p<.05$).

ELISA revealed an increase in the antibody titers after the 2nd months of study in the endemic areas. However, this could be the result of vaccination. The role of carrier animals remained unclear and poorly understood. It could be postulated that latent carriers (if they exist within these populations) remain without shedding for a period of 6 months. The limitations during the field investigation included poor cooperation from the farmers, poor understanding by farmers on the importance of herd health program and the archaic animal management and husbandry.

Pasteurella multocida B:2 isolated from the outbreak investigations were further studied by species specific and type specific multiplex PCR method. The REP-PCR and single primer PCR provided a better trace method for the epidemiological investigation in the disease outbreaks giving many strains that caused the HS. The results of the plasmid profile showed identical patterns in all isolates.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KAJIAN EPIDEMIOLOGI PENYAKIT BERDARAH LEMBU DALAM LEMBU DAN KERBAU DI SEMENANJUNG MALAYSIA

Oleh

KHADAK SINGH BISHT

Jun 2006

Pengerusi: Profesor Abdul Aziz Saharee, PhD

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Kajian retrospektif penyakit hawar berdarah atau "Haemorrhagic Septicaemia" (HS) telah dijalankan melalui kajian soal selidik di 64 daerah di Semenanjung Malaysia. Seribu empat ratus dan lapan puluh sembilan (1489) kematian yang disebabkan oleh HS telah di laporkan daripada Januari 1993 hingga Disember 2003. Maklumat epidemiologi berkenaan dengan corak penyakit, jumlah dan faktor penyebab untuk kejadian cetusan wabak penyakit HS telah dilaporkan. Cetusan wabak penyakit dilaporkan berlaku setiap tahun walaupun banyak langkah pencegahan penyakit telah dijalankan. Daripada sebelas (11) negeri yang diselidik, Terengganu, Kelantan dan Perak didapati sebagai kawasan endemik cetusan wabak HS di Semenanjung Malaysia. Negeri-negeri lain pula bebas daripada penyakit tersebut. Kaedah *time series seasonal decomposition* telah digunakan untuk mengenalpasti corak musim, gaya sekular, kitaran dan ketidakstabilan yang tidak menentu kejadian penyakit HS dan hubunganya dengan corak cuaca hujan, suhu persekitaran dan kelembapan serta

pergerakan haiwan semasa musim perayaan dan semasa vaksinasi juga telah dihuraikan.

Dua ratus empat (204) ekor kerbau dan empat (4) ekor lembu telah disahkan mati disebabkan oleh HS di Batang Padang, Perak pada tahun 2003. Siasatan epidemiologi telah dijalankan semasa cetusan wabak penyakit berlaku, sampel klinikal dikumpul, penternak ditemuduga dan kawasan yang terlibat juga ditinjau. *Pasteurella multocida* B:2 telah dipencilkan dan dikenalpasti daripada darah jantung dan kesatan hidung ke atas haiwan daripada haiwan-haiwan dari kawasan terlibat. Kerbau yang mati di dalam kolam di kawasan ragutan didapati memainkan peranan penting di dalam rebakan penyakit tersebut. Cetusan wabak penyakit adalah disebabkan oleh kombinasi faktor-faktor seperti pengangkutan masuk haiwan pembawa penyakit yang sihat ke kawasan endemik, perubahan cuaca yang nyata serta status imuniti yang rendah dikalangan kelompok haiwan yang terlibat.

Pengkajian sistem ELISA untuk diagnosa penyakit HS telah disahkan kesahihannya berdasarkan pada sampel daripada kumpulan yang dijangkiti dan kumpulan bebas yang penyakit. Daripada kawasan di bawah lengkungan ROC, didapati bahawa ujian tersebut mempunyai kejituan yang tinggi yang membahagikan populasi kepada dua kategori status penyakit. Nilai *cut-off* yang didapati dari analisa ROC (untuk indirect ELISA) telah memberi 86.4% sensitiviti diagnosa dan 84.2% kejituan diagnosa berdasarkan 0.51 poin *cut-off*

Status lembu sebagai haiwan pembawa penyakit organisma *P. mulotcida* B:2 telah diselidik di dalam tiga kelompok lembu di kawasan bukan endemik dan tiga kelompok di kawasan endemik. Lembu-lembu dari setiap ladang yang terpilih telah

dipantau sebanyak 3 ke 5 kali selama tempoh 6 bulan untuk menentukan status pembawa penyakit yang disebabkan oleh organisma pembawa penyakit HS. Sejumlah seratus lapan puluh enam (186) ekor lembu telah di pantau selama kajian ini dijalankan. Pemencilan organisma dilakukan dengan teknik pemencilan dalam mencit dan antibodi serum pula dikesan mengunakan kaedah indirect ELISA. Analisa bakteriologi tidak menunjukkan mana-mana lembu yang dikaji sebagai pembawa organisma penyebab penyakit HS pada sepanjang 6 bulan kajian tersebut/dujalankan.

Walau bagaimanapun, terdapat segelintir daripada populasi yang mempunyai tahap rendah immuniti terhadap penyakit tersebut. Nilai purata OD dari esei- ELISA di kawasan bukan endemik kurang daripada nilai purata OD di kawasan endemik ($p<0.05$). Ujian ELISA menunjukkan peningkatan dalam kadar antibodi berlaku selepas bulan kedua di kawasan endemik tetapi peningkatan ini berkemungkinan disebabkan oleh vaksinasi. Peranan haiwan pembawa penyakit masih tidak jelas dan juga kurang difahami. Maka teori bagi pembawa penyakit yang terpendam adalah jikalau organisma pembawa penyakit wujud di bahagian tonsil lembu di kalangan populasi yang dikaji, organisma tersebut tidak akan disebarkan selama 6 bulan. Beberapa kekurangan/ batasan kajian ini termasuklah faktor-faktor seperti ketiadaan kerjasama dari pihak penternak, ketidakfahaman penternak mengenai kepentingan program kesihatan sejagat kelompok dan juga pengurusan perladangan tradisional yang dipraktikan oleh peternak.

Pemencilan organisma *P. multocida* B:2 yang didapati daripada cetusan rebakan penyakit yang dikaji telah diselidik semula melalui pengkhususan spesies dan

pengkhususan jenis dengan mengunakan kaedah *multiplex* PCR. REP-PCR dan *single primer* PCR adalah kaedah-kaedah pengesan yang lebih baik untuk penyiasatan epidemiologi semasa cetusan wabak bagi pelbagai strain penyebab HS berlaku. Hasil profil plasmid menunjukkan corak serupa dalam semua pencilan organisma.

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I certify that an Examination Committee has met on 14 June 2006 to conduct the final examination of Khadak Singh Bisht on his Doctor of Philosophy thesis entitled "Epidemiology of Haemorrhagic Septicaemia in Cattle and Buffaloes in Peninsular Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree at UPM or other institutions.

Khadak

KHADAK SINGH BISHT

Date: 25/8/06

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